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Comparison of Three Different Methods for Detection of Shiga Toxin-Producing *Escherichia coli* in a Tertiary Pediatric Care Center

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Shiga toxin-producing *Escherichia coli* (STEC) is a well-known cause of sporadic and epidemic food-borne gastroenteritis. A low infectious dose, approximately 10 microorganisms, is sufficient to cause disease that may lead to hemolytic-uremic syndrome. The objective of this study was to compare the performances of an in-house real-time PCR, a commercial enzyme immunoassay (EIA) (Premier EHEC; Meridian Bioscience), and culture on sorbitol MacConkey agar for the detection of STEC in a tertiary care pediatric hospital. Of 632 stool samples tested, 21 were positive for STEC. All were detected by PCR, 6 were detected by EIA, and only 5 O157 STEC isolates were identified by culture. Among the 15 specimens falsely negative by EIA, there were 9 Stx1, 2 Stx2, and 4 Stx1 and Stx2 STEC isolates. The latter group included 2 O157 STEC isolates that would have been missed if only EIA had been performed. To our knowledge, this is the first prospective study performed in a pediatric hospital which demonstrates the superiority of PCR over EIA for the detection of STEC. We conclude that PCR is specific and more sensitive than EIA. PCR should be considered for routine use in clinical settings where molecular detection facilities are available. Its lower limit of detection, equivalent to the infectious dose, is an obvious advantage for patient care and public health surveillance.

Shiga toxin-producing *Escherichia coli* (STEC) is a well-known cause of sporadic and epidemic food-borne gastroenteritis, bloody diarrhea, and hemorrhagic colitis. This infection may lead to hemolytic-uremic syndrome (HUS) in 5 to 15% of cases. This life-threatening condition, more frequent in children, consists of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. HUS is still nowadays the primary cause of acute renal failure in pediatric patients (1, 2).

Shiga toxin-producing *E. coli* strains are defined by the expression of at least one type of Shiga toxin, Stx1 and Stx2, which are encoded on bacteriophages. Stx1 is genetically and structurally similar to the Shiga toxin produced by *Shigella dysenteriae* serotype 1 strains. However, the amino acid sequence of Stx2 is only 56% analogous to that of Stx1 (3–5). Several variants have been described for each type of toxin. They differ by their biological activities and pathogenicities (6). Isolates producing Stx2, alone or in combination with Stx1, are associated with more severe disease and a higher incidence of HUS than those harboring only Stx1 (7, 8). Specifically, Stx2c is recognized to be one of the most virulent variants (9–11). Moreover, other virulence factors have been demonstrated, mainly the intimin gene (*eae*) located on the locus of enterocyte effacement (LEE) pathogenicity island and the enterohemolysin (*ehxA*) gene (9, 10).

More than 200 different serotypes of *E. coli* can produce Shiga toxin, and among them, at least 150 are human pathogens (12–15) In the literature, STEC strains are usually categorized into O157 and non-O157 strains. This categorization is based mainly on historic factors: O157 was the first STEC serotype discovered, is the easiest to identify in a microbiology laboratory, and was thought to be more prevalent and virulent than other serotypes. However, studies from around the world have demonstrated that non-O157 serotypes are at least as prevalent as O157 (16–18). In the United States, epidemiological studies have shown that *E. coli* O26, O103, and O111 are the non-O157 serotypes most frequently encountered (19). Previous studies have also demonstrated that non-O157 STEC strains can cause severe disease similar to that of O157 STEC strains, with bloody diarrhea and HUS, specifically when they produce Stx2 (10, 20). Finally, the recent European O104:H4 outbreak was caused by a typical enteroaggregative *E. coli* strain that has acquired the bacteriophage encoding Stx. This demonstrates that STEC virulence factors encoded on mobile elements could spread among other pathotypes of diarrheagenic *E. coli* and thereafter represent a public health threat (21, 22).

In 2007, FoodNet surveyed all clinical laboratories that are part of their network to determine their diagnostic testing practices for the identification of STEC. Hoefer et al. reported the results: only 11% performed an Stx enzyme immunoassay (EIA) either alone or in combination with culture. It was a 5% improvement since 2003 (23). Similar results were obtained by Stigi et al.: in 2011, approximately 65% of Washington State microbiology laboratories were limiting the identification of STEC to the O157 serotype (17). Laboratories that test for non-O157 strains use EIAs (17, 23). Molecular detection is still not routinely used in clinical settings, likely because no commercial assays approved by the FDA for the diagnosis of human STEC infections are available.

In 2009, the CDC published guidelines stipulating that clinical laboratories should perform simultaneously an assay that will detect either the Shiga toxins directly or the genes encoding them and a selective culture for O157 STEC (24). The performance of both techniques at the same time enables laboratories to rapidly detect both O157 and non-O157 STEC isolates. It also ensures prompt outbreak investigations by public health authorities. In Canada, laboratories are required by law to report all STEC infections and not only those caused by O157 STEC isolates. This reg-

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ulation warrants the implementation by clinical laboratories of assays that enable this surveillance.

In this study, we evaluated the performances of an in-house real-time PCR assay and of the Premier EHEC toxin EIA (Meridian Bioscience Inc., Cincinnati, OH) in a clinical microbiology laboratory of a tertiary care pediatric center. We also compared their performances with that of culture on sorbitol MacConkey agar (SMAC), which was already performed in our hospital as part of our routine workup for the detection of enteric bacterial pathogens.

MATERIALS AND METHODS

Study description. This study took place at CHU Sainte-Justine (Montreal, Canada), a mother-child university health center with 484 beds and an average of 65,000 emergency room visits annually. From 1 June to 30 September 2009, 632 consecutive stool samples from pediatric patients (aged <18 years) submitted to the microbiology laboratory for the detection of bacterial enteric pathogens were included in the study. Samples were plated onto SMAC for the detection of O157 STEC. In addition, for each sample, 200 μl of watery stool or a pea-size amount of stool was inoculated into 5 ml MacConkey broth and incubated at 35°C for 15 to 24 h. The enrichment broth was then tested for the presence of Shiga toxins by the Premier EHEC assay (Meridian Bioscience Inc., Cincinnati, OH) and by real-time PCR for the detection of the stx_1 and stx_2 genes. All positive samples were sent to the Alberta Provincial Public Health Laboratory (Edmonton, Alberta, Canada) for confirmation. Clinical charts of all positive patients were reviewed.

Conventional culture. All stool samples were plated onto Columbia blood agar, Hektoen enteric agar, MacConkey agar, Skirrow agar, SMAC, and *Yersinia* selective agar for the detection of *Aeromonas* sp., *Campylobacter* sp., O157 STEC, *Plesiomonas* sp., *Salmonella* sp., *Shigella* sp., and *Yersinia* sp., as routinely recommended (25). For O157 STEC detection, the SMAC plates were incubated at 35°C for 16 to 24 h and then examined for non-sorbitol-fermenting colonies. Three colorless colonies were tested for the presence of the O157 lipopolysaccharide antigen by latex particle agglutination (Remel, Lenexa, KS). Colonies positive for O157 were then identified by conventional biochemical testing, using API 20E strips (bioMérieux, St-Laurent, Canada).

Enzyme immunoassays. The Premier EHEC assay is a microwell EIA for the detection of Shiga toxins 1 and 2 without the differentiation of the toxins. For all samples, 50 μl of incubated MacConkey broth was tested with this assay, according to the manufacturer's instructions. Following toxin detection by using the Premier EHEC kit, the Shiga toxin type was determined by using an immunochromatographic rapid test, the ImmunoCard Stat! EHEC assay (Meridian Bioscience Inc.), according to the manufacturer's instructions. The analytical specificities and sensitivities of both assays were respectively evaluated with a panel of organisms commonly isolated from feces and different dilutions of O157 STEC (see Table 2).

Real-time PCR. For each stool sample, bacterial DNA was extracted by using the QIAamp DNA blood minikit (Qiagen, Mississauga, Canada), according to the manufacturer's instructions. Briefly, 200 μl of incubated MacConkey broth was used for extraction, and DNA was eluted in 100 μl of elution buffer. The primers and MGB (minor grove binder) probes for stx_1 and stx_2 real-time PCR were chosen for specific regions of each gene (Table 1) by using Primer Express software (Applied Biosystems, Foster City, CA). To detect PCR inhibition, primers and a probe were also designed in a plasmid (pARAB) containing part of the *Arabidopsis thaliana* chlorophyll synthetase gene (UHN Microarray Center, University of Toronto, Toronto, Ontario, Canada). The specificities of all primers and probes were confirmed by BLAST analysis (26). The real-time PCR assay was performed with an AB7500 instrument (Applied Biosystems) as a triplex reaction for the detection of stx_1 (VIC dye), stx_2 (6-carboxyfluorescein [FAM] dye), and the pARAB plasmid (NED dye), using the following amplifications conditions: 95°C for 15 min, 35 cycles of 95°C for 15 s, and 60°C for 40 s. The reactions were performed in a final volume of 25 μl, using the QuantiTect Multiplex PCR NoROX kit (Qiagen), with ROX (Invitrogen, Carlsbad, CA) added at 50 nM, primers and probes at the optimal concentrations (Table 1), 130 copies of the pARAB plasmid, and 5 μl of extracted DNA. In each run, samples were tested in triplicates. Positive-control (O157 STEC genomic DNA positive for stx_1 and stx_2) and nontemplate control (PCR-grade water) samples were included. Cross-reactivity and sensitivity studies were performed with the same panel of organisms and concentrations of O157 STEC used for immunoassays (Table 2). All positive samples were sent to the Alberta Provincial Public Health Laboratory for confirmation with a real-time PCR assay that used different primers and probes (27).

TABLE 1 Oligonucleotides used in the real-time PCR assay

Target	Primer or probe sequence (5′–3′)	Final concn (nM)	Amplicon size (bp)
stx_1	GACGCAGTCTGTGGCAAGAG	300	69
	TGCCGAAAACGTAAAGCTTCA	900	
	VIC-ATGTTACGGTTTGTTACTGTGA	300	
stx_2	CAACGGACAGCAGTTATACCACTC	300	76
	TTAACGCCAGATATGATGAAACCA	500	
	FAM-AATGCAAATCAGTCGTCACT	300	
pARAB[a]	TGTGGGCAGGGCATACC	50	58
	AGCAATGATCCTCCCAAAGC	300	
	NED-CCCACTGTCTTCTATC	100	

[a] Plasmid containing part of the *Arabidopsis thaliana* chlorophyll synthetase gene (UHN Microarray Center, University of Toronto).

STEC isolation. All positive samples were subcultured from MacConkey broth onto MacConkey agar plates, and a maximum of 100 colonies were tested for the presence of stx_1 and/or stx_2 by real-time PCR. All identified STEC isolates were sent to the National Microbiology Laboratory (Winnipeg, Canada) for typing of the O and H antigens.

RESULTS

Performances of PCR, Premier EHEC EIA, and SMAC culture. **(i) Analytical specificity.** No cross-reactivity or false-positive result occurred with any assay (Table 2). PCR and ImmunoCard Stat! EHEC properly detected the stx_1 and stx_2 genes and toxins, respectively.

(ii) Analytical sensitivity. The level of detection for the ImmunoCard Stat! EHEC assay was 10^7 CFU/ml of O157 STEC, and that for the Premier EHEC EIA was 10^6 CFU/ml. PCR was able to detect each target in a suspension of 10^2 CFU/ml (Table 2).

(iii) Performance with clinical specimens. During the study period, a total of 632 stool samples from 430 different pediatric patients were tested. Among them, 21 stool samples, representing 12 patients, were positive for STEC by at least one assay: PCR identified all of them, and 6 were detected by the Premier EHEC EIA. SMAC culture retrieved 5 STEC O157 isolates; 2 of them were missed by the EIA. Table 3 shows the performances of the different assays, according to the toxin type present in the sample. All samples positive by PCR were confirmed at the Alberta Provincial Public Health Laboratory by a second real-time PCR assay. Among the positive patients, 7 had submitted more than one stool sample. No discordant results occurred with PCR. Discordant results with the EIA were observed for three patients.

Serotyping. Among the positive specimens, 16 Shiga toxin-producing strains were isolated by subculturing of MacConkey

TABLE 2 Organisms tested for analytical sensitivity and specificity

Species	Origin[a]	Result by test			
		Real-time PCR			
		stx_1	stx_2	Premier	ImmunoCard Stat!
Bacteroides fragilis	ATCC 25285	−	−	−	−
Campylobacter coli	Clinical isolate	−	−	−	−
Campylobacter jejuni	ATCC 33291	−	−	−	−
Candida albicans	ATCC 10231	−	−	−	−
Citrobacter amalonaticus	Clinical isolate	−	−	−	−
Citrobacter braakii	Clinical isolate	−	−	−	−
Citrobacter freundii	Clinical isolate	−	−	−	−
Clostridium difficile	ATCC 9689	−	−	−	−
Corynebacterium renale	ATCC 19412	−	−	−	−
Enterobacter cloacae	ATCC 13047	−	−	−	−
Enterococcus faecalis	ATCC 29212	−	−	−	−
Escherichia coli	ATCC 25922	−	−	−	−
Escherichia coli	ATCC 35218	−	−	−	−
Escherichia coli O157 (10^7 CFU/ml)	Clinical isolate	+	+	+	ST1&2[b]
Escherichia coli O157 (10^6 CFU/ml)		+	+	+	−
Escherichia coli O157 (10^5 CFU/ml)		+	+	−	−
Escherichia coli O157 (10^3 CFU/ml)		+	+	−	−
Escherichia coli O157 (10^2 CFU/ml)		+	+	−	−
Escherichia coli O157 (10 CFU/ml)		−	−	−	−
Fusobacterium nucleatum	ATCC 25506	−	−	−	−
Hafnia alvei	Clinical isolate	−	−	−	−
Klebsiella oxytoca	ATCC 700324	−	−	−	−
Klebsiella pneumoniae	ATCC 700603	−	−	−	−
Lactobacillus acidophilus	ATCC 4356	−	−	−	−
Listeria monocytogenes	Clinical isolate	−	−	−	−
Morganella morganii	Clinical isolate	−	−	−	−
Peptostreptococcus anaerobius	ATCC 27337	−	−	−	−
Proteus mirabilis	ATCC 35659	−	−	−	−
Pseudomonas aeruginosa	ATCC 27853	−	−	−	−
Salmonella enterica	ATCC 14028	−	−	−	−
Salmonella enterica serovar Typhi	Clinical isolate	−	−	−	−
Shigella flexneri	ATCC 12022	−	−	−	−
Shigella sonnei	ATCC 29930	−	−	−	−
Staphylococcus aureus	ATCC 29213	−	−	−	−
Yersinia enterocolitica	ATCC 9610	−	−	−	−

[a] ATCC, American Type Culture Collection.
[b] ST1&2, positive for Shiga toxins 1 and 2.

broth. A pathogenic strain was isolated from each EIA-positive sample. The National Microbiology Laboratory determined that 5 were O157 STEC and that 11 were non-O157 strains. Among the 7 *E. coli* isolates producing only Stx1, there were two O26:H11, two O49 (H10/H$^-$), one O8:H9, one O73H29, and one O111:H$^-$ isolates. The 2 Stx2-producing strains were O153 (H9/H$^-$) isolates. Finally, among the seven Stx1- and Stx2-positive isolates, there were five O157:H7 isolates (recovered by SMAC culture), one O3:H9 isolate, and one O86:H$^-$ isolate.

Other enteric bacterial pathogens. Of the 632 stool samples tested, 75 (11.9%) were positive by culture for an enteric bacterial pathogen other than STEC (Table 4). *Salmonella* sp. was the most frequently encountered pathogen, followed by *Campylobacter* sp.

Patients. To ascertain the clinical specificity of the assays, we

TABLE 3 Performances of assays according to toxin type

Assay	No. positive							
	Stx1		Stx2		Stx1 and Stx2		Total	
	Specimens	Patients	Specimens	Patients	Specimens	Patients	Specimens	Patients
PCR	12	8	2	1	7	3	21	12
EIA Premier	3	2	0	0	3	2	6	4
ImmunoCard	3	2	0	0	1	1	4	3
SMAC	0	0	0	0	5	3	5	3

TABLE 4 Stool culture results for common enteric pathogens ($n = 632$ for 430 patients)

Pathogen	No. (%) of isolates	No. (%) of patients
Salmonella sp.	40 (6.3)	22 (5.1)
Campylobacter sp.	21 (3.3)	13 (3.0)
O157 and non-O157 STEC	21 (3.3)	12 (2.8)
O157 STEC	5 (0.8)	3 (0.7)
Aeromonas sp.	8 (1.3)	7 (1.6)
Shigella sp.	4 (0.6)	2 (0.5)
Yersinia sp.	2 (0.3)	1 (0.2)

retrospectively reviewed the medical charts of the 12 positive patients. All patients but one were symptomatic. Two patients required hospitalization; they were infected by O157 STEC strains producing both Stx1 and Stx2. The first patient was detected by all assays. The second patient submitted 3 specimens; all were positive by PCR but negative by EIA. Culturing on SMAC recovered O157 STEC in one sample. No patient developed HUS. Otherwise, eight patients had bloody diarrhea (4 identified by EIA and 3 identified by SMAC culture). One of the patients, with infection detected only by PCR, underwent a diagnostic colonoscopy.

Three patients were infected by more than one enteric pathogen. One was coinfected with *Campylobacter jejuni* and *E. coli* O49:H10/H$^-$. The second patient had 2 different STEC serotypes in his stool samples (*E. coli* O111:H$^-$ and O73:H29), and the third patient harbored 3 different strains (O157:H7, O86:H$^-$, and O3:H9). Interestingly, the twin sister of the third patient was infected with a different strain (O153), despite the fact that they shared similar risk factors.

DISCUSSION

Traditionally, only O157 STEC was routinely identified in clinical diagnostic laboratories. Since most experts agree that this practice leads to an underestimation of STEC cases, the true prevalence remains largely unknown.

The purpose of our study was to compare and validate the performances of a real-time PCR assay, an enzyme immunoassay, and culture on SMAC for the detection of STEC in our pediatric population. In our laboratory, EIA identified only 29% of the positive samples, or 33% of the infected patients.

PCR performed better than the EIA that we tested, likely due to its higher analytical sensitivity. Indeed, our PCR level of detection is 10^4 times lower than that of the Premier EHEC EIA. A similar analytical sensitivity of 10^6 CFU/ml for Premier EHEC was previously reported by Willford et al. in a study that evaluated three commercially available EIA kits for the detection of STEC (28). The difference of 4 $\log_{10}$ units between the nucleic acid amplification test (NAAT) and EIA is also concordant with results obtained previously by Chui et al. (29). However, in the latter study, the performance of the EIA in detecting Shiga toxin in stool samples was similar to that of PCR: it missed only 2 of the 21 positive specimens. This difference might be explained by a reference bias. The Alberta Provincial Public Health Laboratory is a reference center that likely receives specimens from sicker patients who may have a higher bacterial load, at a count detectable by EIA. In our study, the lower sensitivity of the EIA led to 15 false-negative samples from 8 different patients. False-negative specimens were associated with a lower inoculum; EIA did not detect samples with a real-time PCR crossing threshold (C_T) of greater than 20 (data not shown). Moreover, considering that fewer than 100 bacteria may cause gastrointestinal disease and that the bacterial load decreases rapidly during the period of disease (3, 30–33), the better sensitivity of PCR represents a clear advantage that must be taken into account when implementing an assay for the detection of STEC. Also, according to our retrospective review of medical charts, severe clinical manifestations were seen even in patients with low bacterial loads, reinforcing the advantage of a sensitive assay.

During the study period, the EIA failed to detect two O157 isolates that were recovered by culture on SMAC and PCR. One of these patients had a severe clinical course, requiring hospitalization. This well-known limitation of the Premier EHEC EIA (34, 35) justifies the recommendation of performing culturing on SMAC simultaneously with a second assay targeting Shiga toxins or the genes encoding them.

The identification and reporting of the toxin type to clinicians are considered to be useful clinical information, with Stx2 recognized as being more virulent than Stx1 (7, 8). The Premier EHEC EIA has the disadvantage of relying on a second test to differentiate the type of toxin present in the sample. Performing the ImmunoCard Stat! assay is expensive, requires additional personnel time, and delays the emission of a final report. Given our increasing knowledge of STEC pathogenicity, assays that distinguish Stx subtypes and identify STEC virulence factors need to be developed and implemented in clinical practice. Patient management would likely be modified if this information was reported to clinicians and public health authorities.

The isolation of STEC strains is important for epidemiological purposes; a pure culture is needed for serotyping. In our study, 16 STEC strains were isolated from 8 patients. The difficulty in recovering STEC strains was previously reported by different authors (27, 29, 36). This may be explained by the freeze-and-thaw effect killing or inhibiting the growth of pathogens. It is also possible that the pathogen's inoculum size in some samples was too low for growth on an agar plate. Also, the performance of PCR on multiple colonies for each sample plated onto MacConkey agar is a time-consuming and demanding task with several limitations: STEC may easily have been missed, even if many colonies were tested for each sample. Because all our positive samples were confirmed by the Alberta Provincial Public Health Laboratory, it is unlikely that they were false-positive samples.

Among the 16 isolated STEC strains, 11 were non-O157 STEC isolates. Although we were not able to identify an STEC isolate from each stool sample, this result suggests that the majority of STEC infections in our population are caused by non-O157 serotypes. No non-O157 serotype predominated during our study period, suggesting the role of sporadic infection rather than an outbreak. However, this conclusion is limited by the fact that we are not a reference center. With the exception of O111 and O26, other non-O157 serotypes that we found do not seem to be routinely identified in North America. This may reflect the changing and poorly known epidemiology of non-O157 STEC infections (17).

Coinfections with other enteric bacterial pathogens were detected in three patients, one with simultaneous *Campylobacter jejuni* infection and the other two with multiple serotypes of Shiga toxin-producing *E. coli*. This finding was previously described by many studies (37–40). Among them, Hedican et al. performed a sentinel surveillance of STEC infections in Minnesota and found 22 coinfected patients among 302 STEC-positive patients (40).

This is likely due to a common fecal-oral route of transmission of enteric pathogens and potential massive contamination when basic rules of hygiene are broken.

The expected clinical impact of our assay is difficult to ascertain, given our small sample size. This project was a diagnostic assay validation study; therefore, clinicians were not aware of EIA or PCR results during the study period. Nonetheless, a review of the medical charts revealed that all but one of our patients were symptomatic. The severity of symptoms (hospitalization and bloody diarrhea) seemed to be associated with Stx2-producing strains, more precisely with O157 STEC strains. This observation is concordant with the medical literature but biased in our study by the fact that three out of the four patients harboring Stx2-producing strains were infected with O157 STEC. Since the introduction of PCR for the routine detection of STEC, we are now prospectively collecting data on identified cases to better define their clinical presentations and outcomes.

Little is known about the persistence of STEC pathogens in the gastrointestinal tract or about the prevalence of asymptomatic carriage. With the advent of molecular detection, clinicians will likely increasingly be faced with laboratory results that are discordant with the patient's clinical presentation. It will also be necessary to reinforce the importance of prescribing the test only when the pretest probability is high and in situations for which it has been validated, i.e., the detection of community-acquired diarrhea.

To our knowledge, ours is the first prospective study to compare head-to-head an in-house real-time PCR and a commercial EIA in a clinical pediatric laboratory setting. The performance of PCR in addition to culture increased our STEC detection rate by 320%, while the addition of the EIA instead increased it by only 60%. In a recent point-counterpoint published in the *Journal of Clinical Microbiology*, authors arguing against universal screening for Shiga toxin based their reasoning on cost-effectiveness and presumed low-prevalence arguments. They nuanced their opinion, stipulating that each laboratory should realize a prevalence study in their own population and consider performing universal screening for Shiga toxin if the prevalence of STEC is as high as that of other enteric pathogens (41). In our study, the prevalence of STEC was 3,3%, which makes it the third most common bacterial pathogen in our pediatric population, similar to *Campylobacter* sp. In short, the epidemiological and PCR performance data obtained in this study justify the implementation cost of a molecular assay able to detect all STEC serotypes in our clinical laboratory and may lead to increasing numbers of microbiology laboratories performing these assays to detect STEC.

Conclusion. This study highlights the superiority of molecular assays for the detection of STEC. It also demonstrates the high prevalence of non-O157 STEC strains in our epidemiology, supporting the implementation of routine screening for Shiga toxin-producing *E. coli*. Our results are concordant with current CDC recommendations. Clearly, we would underestimate the prevalence of STEC infections if only O157 cultures are performed. In our hospital, we have decided to implement PCR in the routine for each stool sample sent for enteric bacterial pathogen detection. Due to its increased sensitivity, we suggest that where resources and facilities are available, molecular detection should be favored instead of an enzyme immunoassay. Further work is needed to better define the clinical specificity of PCR, the duration of STEC shedding after an infection, and the prevalence of asymptomatic carriers in order to help clinicians and guide public health authority recommendations.

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